Magna Scientia Advanced Biology and Pharmacy

eISSN: 2582-8363

Cross Ref DOI: 10.30574/msabp

Journal homepage: https://magnascientiapub.com/journals/msabp/

(RESEARCH ARTICLE)

Check for updates

Antimicrobial and antioxidant activities of extract and fractions of the African yam bean (*Sphenostylis stenocarpa*) Seed

Cynthia A. Onwughalu [1,*], Chiamaka B. Ugwu [2], Ugochukwu M. Okezie [3], Chukwuebuka C. Onwuzuligbo [1] and Felix A. Onyegbule [1]

[1] *Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe, University, Awka, Anambra State, 5025, Nigeria.*
[2] *Department of Pharmacology and Toxicology, Nnamdi Azikiwe, University, Awka, Anambra State, 5025, Nigeria.*
[3] *Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe, University, Awka, Anambra State, 5025, Nigeria.*

Magna Scientia Advanced Biology and Pharmacy, 2025, 14(01), 008-015

Publication history: Received on 24 November 2024; revised on 05 January 2025; accepted on 08 January 2025

Article DOI: https://doi.org/10.30574/msabp.2025.14.1.0012

Abstract

The African yam bean (AYB) (*Sphenostylis stenocarpa*) is a medicinal plant that has poorly been researched on. It is a member of the leguminous crop belonging to the Fabaceae family. AYB's potential therapeutic qualities make it a candidate for drug discovery. This study was carried out to ascertain the pharmacological properties of the extracts. Ethanolic extract and fractions of AYB seed were tested on selected microorganisms: *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella* spp*, Escherichia coli, and Candida albicans* using agar well diffusion assay, and evaluated for their potential to scavenge free radicals adopting Ferric reducing antioxidant power (FRAP) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assays, and finally subjected to phytochemical analysis using standard procedures. The crude extract and fractions produced dose-dependent and broad-spectrum antimicrobial activity. The inhibition zones and minimum inhibitory concentration also varied across the test organisms having 2 - 6 mm and 12.5 – 100 mg/ml respectively. The ethylacetate fraction was observed to be the most active extract. The crude extract and fractions produced a moderate capacity to scavenge free radicals by generating Diphenylpicrylhydrazine (reduced) through the conversion of DPPH as indicated. Phytoactive constituents such as alkaloids, tannins, flavonoids, steroids, terpenoids, and saponins detected in the crude extract and fractions may be responsible for the pharmacological activities recorded. The result of this study further validates the possibility of identifying a lead in the extract of AYB with therapeutic potentials.

Keywords: African yam bean; *Sphenostylis stenocarpa*; Antimicrobial; Dose-dependent; Antioxidant

1. Introduction

In developing countries, large numbers of the world's population are unable to afford pharmaceutical drugs and they continue to use their systems of indigenous medicine that are mainly plant-based [1]. There is the basic supposition that any plant possessing clinical effectiveness must contain an active principle that can completely replace the plant extract. There is a consistent increase in the use of products of medicinal origin which is believed to be due to the observed efficacy with about 80% of the world's population relying on herbal medicinal products as a primary source of healthcare [2]. The African yam beans (*Sphenostylis stenocarpa* L.) (AYB) are an important member of legumes in tropical regions used in the propagation of good health [3]. The herbs used in the preparation of herbal products are seen by the communities as an integral part of their culture [3, 4, 5]. This legume has been reported to be of importance

*Corresponding author: Cynthia A. Ibida

in the management of chronic diseases like diabetes, hypertension, and cardiovascular diseases because of its high dietary fibre content [6]. It is eaten roasted as groundnut or boiled and blended with ingredients like oil, pepper, onions and salt. Previous studies have reported that AYB anti-diabetic activity [7]; crude extract of the powdered seeds has been reported to possessanti-inflammatory, antidiabetics, antioxidant,hepato-renal and hematological activities [8]. Currently, complementary and alternative medicines (CAMs) are gradually becoming mainstream as a result of their use [9, 10, 11]. The consistent patronage/use of herbal products by the developed countries is believed to be due to their belief that it will promote healthier living.

Antioxidants are known for their unique capacity to halt chronic and degenerative diseases through their radical scavenging potentials to deactivate the activities of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which are the major cause of these diseases [12]. This has led to continued use of and consumption of antioxidant-rich foods or fruits. Owing to the high contents of important phytochemicals with several health benefits such as antioxidants and antimicrobials, legumes have been demonstrated to be considered promoters of good health [13]. The African yam beans (*Sphenostylis stenocarpa* L.) are an important member of legumes in tropical regions used in the propagation of good health [14].

Information on the antimicrobial and antioxidant properties of *Sphenostylis stenocarpa* L. (AYB) is scanty. Therefore, the aim of this study is to evaluate the antimicrobial and antioxidant potentials of methanol crude extract and fractions of n-hexane, butanol, ethyl acetate and water.

2. Materials and Methods

2.1. Extraction and preparation of *Plant material*

Fresh powdered seeds of *Sphenostylis sternocarpa* (African yam bean) weighing 500 g were extracted with ethanol in a Soxhlet apparatus [15]. The ethanolic crude extract obtained was partitioned into ethyl acetate, butanol, n-hexane, and water fractions. A stock concentration of 200 mg/mL of each of the extracts was made by weighing 400 mg of the crude and each fraction and reconstituting in 2 mL dimethyl sulfoxide (DMSO). Thereafter, a two-fold serial dilution was made from each of the stock concentrations to get graded concentrations (100, 50, 25, and 12.5 mg/mL) of each of the extracts.

2.2. Bioassay

2.2.1. Antimicrobial assay

The antimicrobial assay for the crude and each of the fractions was carried out using the agar well diffusion assay as described by [16]. A standardized 0.5 McFarland suspension of each test organism was done using sterile water and inoculated onto previously sterilized Mueller-Hinton Agar plates (diameter: 90 mm) while the standardized fungal culture was inoculated onto Sabouraud dextrose agar plates. A maximum volume of 80 µl of each extract dilution was transferred into it corresponding well made with a cork borer having a diameter of 5mm. Ciprofloxacin (5.6 mg/mL) and fluconazole (4.8 mg/mL) served as the positive controls against the bacteria and fungi organisms respectively. The cultures were incubated at 37°C for 24 h (for bacteria) and 25°C for 48 h (for fungi) plates respectively. The antimicrobial potential for each extract was determined by measuring the zone of inhibition around each well (excluding the diameter of the well). The experiment was done in triplicate against each organism. Each extract was tested against all the bacterial and fungal strains respectively.

2.2.2. Determination of Minimum Inhibitory Concentration (MIC)

The method described by *the European Committee for Antimicrobial Susceptibility Testing* (EUCAST) [17] was adopted with slight modifications. The Minimum Inhibitory Concentration (MIC) of the extracts was determined for each of the test organisms in triplicate Petri dishes. Here, the agar dilution method was adopted.

Stock solutions of 4000 mg/ml of the various extracts were prepared. Then, two-fold serial dilutions were made to get 2000, 1000, 500, 25, and 125 mg/mL thereafter 10-fold dilutions of each of the concentrations were made using 9 mL sterile molten agar this was allowed to solidify. The microbial inoculums which have been standardized to 0.5 McFarland turbidity are streaked on the agar appropriately. The plates are incubated at 37°C for 24 hrs for the bacteria plates.

After incubation, the plates were examined for microbial growth by checking for growths using a plus sign (+) indicating growth while a negative sign (-) indicates no growth. * indicates no MIC was carried out because there was no antibacterial activity.

2.3. Antioxidant assay

2.3.1. Ferric reducing antioxidant power (FRAP) assay:

FRAP assay was carried out following the method described by Erhirhie et al [18]. Two hundred and fifty microlitre (0.25 ml) of various fold dilutions of samples as well as 7.82, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 µg/ml ascorbic acid were mixed with 0.625 ml of phosphate buffer and 0.625 ml of 1% potassium ferricyanide [K_3FeCN_6]. The mixtures were heated at 50^0C for twenty minutes. Then, 0.625 ml of 10% trichloroacetic acid (TCA) was added and the mixtures were centrifuged at 3000 rpm for 5 minutes. From the upper layer, 0.625 ml was pipetted and mixed with 0.625 ml of distilled water and 0.125 ml of 0.1% (w/v) ferric chloride ($FeCl_3$) solution. Absorbance of the mixture were measured at 700 nm against air using a Spectrophotometer. Ascorbic acid was used as standards. Tests were performed in triplicates.

Percentage inhibition was calculated using the formula below.

% Inhibition = (Absorbance of sample – Absorbance of blank) x 100

For ascorbic acid, a graph of percentage inhibition against concentration was plotted and the effective concentration (EC_{50}) was extrapolated using an equation.

High optical density (absorbance) values indicate high FRAP activity.

2.3.2. Antioxidant Assay: 2, 2-diphenyl -1- picrylhydrazyl (DPPH) scavenging (DPPH)

The propensity of the fungal crude extract to inactivate free radicals such as 2,2-diphenyl-1-picryl hydrazyl (DPPH) was determined using a previously described procedure Okezie *et al*., [19]. In order to prepare a concentration of 100 µg/mL of both the fungal extract and positive control (quercetin), 300 µg of each was weighed and dissolved in 3 mL of methanol. A reaction mixture comprising 0.25 mL of the stock, 0.25 mL of DPPH (0.6 mMol) and 2 mL of methanol solution was formed. These were incubated at 27 °C for 30 min**.** Then, the absorbance which is a measurement of the antioxidant capacity was determined at 517 nm using a UV-vis spectrophotometer (model 721, ANENG, China). The measurement for the extract and control was carried out in triplicate. The capacity to disrupt free radicals were calculated using the formula below and expressed as percentage inhibition.

$$\%\ Inhibition = \frac{A0 - A1}{A0} x \frac{100}{1}$$

where A0: Absorbance of blank; A1: Absorbance of test sample

2.4. Phytochemical Screening

Phytochemical analyses on the aqueous extracts of the powdered samples of African Yam Bean (*Sphenostylis stenocarpa L*) were carried out using standard procedures to identify the phytochemical constituents as described by Harborne, [20]; Trease and Evans, [21]; Sofowora, [22].

2.5. Statistical analysis

Measurements were done in triplicate (n = 3) and the results expressed as mean ± standard deviation.One way analysis of variance (ANOVA) and SPSS (version 20) was used as the statistical program.

3. Results and Discussion

The antimicrobial activities demonstrated by the crude extract and fractions of AYB are presented in Tables 1 -5. The observed activities by the extracts demonstrates their diverse biological potentials.

Table 1 Antimicrobial activity of Crude extract against the pathogens

Concentration (mg/mL)	**Test organisms / inhibition zone diameter (mm)**				
	P. aeruginosa	***S. aureus***	***E. coli***	***Salmonella***	***C. albicans***
100	4±0	3±0	0±0	0±0	4±0.7
50	4±0.7	2±0	0±0	0±0	4±0.7
25	0±0	0±0	0±0	0±0	2±0
12.5	0±0	0±0	0±0	0±0	0±0
6.25	0±0	0±0	0±0	0±0	0±0
Pos. control	15	0	0	0	0

P. aeruginosa: Pseudomonas aeruginosa; S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; Salmonella spp; *C. albicans: Candida albicans*; Pos Ctrl: Positive controls: Ciprofloxacin 5.6 mg/mL, Fluconazole 4.8 mg/mL

Table2 Antimicrobial activity of Ethyl acetate fraction against the pathogens

Concentration (mg/mL)	**Test organisms / inhibition zone diameter (mm)**				
	P. aeruginosa	***S. aureus***	***E. coli***	***Salmonella***	***C. albicans***
100	0±0	3±0	2±0	3.5±0.7	5±0
50	0±0	0±0	0±0	3±1.2	4.5±0.7
25	0±0	0±0	0±0	2.5±0.7	4±0
12.5	0±0	0±0	0±0	0±0	3±0
6.25	0±0	0±0	0±0	0±0	0±0
Pos. control	15	0	0	0	0

P. aeruginosa: Pseudomonas aeruginosa; S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; Salmonella spp*; C. albicans: Candida albicans;* Pos Ctrl: Positive controls: Ciprofloxacin 5.6 mg/mL, Fluconazole 4.8 mg/mL

Table 3 Antimicrobial activity of Butanol fraction against the pathogens

Concentration (mg/mL)	**Test organisms / inhibition zone diameter (mm)**				
	P. aeruginosa	***S. aureus***	***E. coli***	***Salmonella***	***C. albicans***
100	3±0	3±0	4±0	0±0	6±0
50	0±0	2.5±0.7	2±0	0±0	5±0
25	0±0	2±0	0±0	0±0	4.5±0.7
12.5	0±0	0±0	0±0	0±0	3±0
6.25	0±0	0±0	0±0	0±0	0±0
Pos. control	15	0	0	0	0

P. aeruginosa: Pseudomonas aeruginosa; S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; Salmonella spp; *C. albicans: Candida albicans*; Pos Ctrl: Positive controls: Ciprofloxacin 5.6 mg/mL, Fluconazole 4.8 mg/mL

Table 4 Antimicrobial activity of n-hexane fraction against the pathogens

Concentration (mg/mL)	**Test organisms / inhibition zone diameter (mm)**				
	P. aeruginosa	***S. aureus***	***E. coli***	***Salmonella***	***C. albicans***
100	0±0	2±0	0±0	4±0	4±0
50	0±0	0±0	0±0	0±0	2±0.7
25	0±0	0±0	0±0	0±0	2±0
12.5	0±0	0±0	0±0	0±0	0±0
6.25	0±0	0±0	0±0	0±0	0±0
Pos. control	15	0	0	0	0

P. aeruginosa: Pseudomonas aeruginosa; S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; Salmonella spp*; C. albicans: Candida albicans*; Pos Ctrl: Positive controls: Ciprofloxacin 5.6 mg/mL, Fluconazole 4.8 mg/mL

Table 5 Antimicrobial activity of Aqueous fraction against the pathogens

Concentration (mg/mL)	**Test organisms / inhibition zone diameter (mm)**				
	P. aeruginosa	***S. aureus***	***E. coli***	***Salmonella***	***C. albicans***
100	4±0	3±0	2±0	0±0	0±0
50	0±0	2±0	2±0	0±0	0±0
25	0±0	0±0	0±0	0±0	0±0
12.5	0±0	0±0	0±0	0±0	0±0
6.25	0±0	0±0	0±0	0±0	0±0
Pos. control	15	0	0	0	0

P. aeruginosa: Pseudomonas aeruginosa; S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; Salmonella spp; *C. albicans: Candida albicans*; Pos Ctrl: Positive controls: Ciprofloxacin 5.6 mg/mL, Fluconazole 4.8 mg/mL

Table 6 Minimum inhibitory concentration

Extract	**Test organism / Concentration (mg/mL)**				
	P. aeruginosa	***S. aureus***	***E. coli***	***Salmonella***	***C. albicans***
crude	100	200	>200	>200	100
Ethyl acetate	>200	200	200	100	50
n-hexane	>200	200	>200	200	200
Butanol	200	200	200	>200	50
Aqeous	200	200	200	>200	>200

Table 7 Antioxidant activities

S/n	Extract/Fraction	FRAP activity: EC_{50} (ug/ml)	DPPH activity: IC_{50}(µg/mL)
1	Crude extract	7298.71	57.5
2	n-hexane fraction	6426.75	62
3	Ethyl acetate fraction	828.81	60
4	Butanol fraction	7194.86	94.9
5	Aqueous fraction	6222.75	312.5
6	Ascorbic acid	87.60	48

The lower the EC_{50}, the higher the antioxidant activity. EC_{50} of each sample was calculated from the equation of the graph of percentage inhibition against concentration.

Table 8 Phytochemical analysis

Extract/Fraction	Phytoactive Constituent					
Crude extract	Al	Ta	Fl	St	Te	Sa
n-hexane fraction	+	+	+	+	+	+
Ethyl acetate fraction	+++	+	++	+	+++	++
Butanol fraction	++	+	+++	+	+	+
Aqueous fraction	+	+	+++	+	+	++

Key: Al: alkaloids, Ta: tannins, Fl: flavonoids, St: steroids, Te: terpenoids, Sa: saponins

The preliminary antimicrobial evaluations of the *Sphenostylis sternocarpa* crude extract and fractions in Table 1-5 reveal that all the extracts demonstrated broadspectrum antimicrobial activity against at least one Gram-positive and Gram-negative bacterium. The observed activities were concentration-dependent as the zone of inhibition was observed to increase with the concentration for the crude extract and the fractions. At the tested concentrations which ranged between 6.25 – 100 mg/mL, the growth inhibitory potentials of the crude extract and the fractions against the different test organisms varied as seen by their varying degree of inhibition zones produced which ranged between 2 – 4mm (*S. aureus, P. aeruginosa, and E. coli*), 2.5 – 4 mm (*Salmonella* spp), and 2 – 6 (*C. albicans*). Similarly, Eruemulor, [23] observed varying zones of inhibition among the tested microorganisms; *Aspergillus niger* (19.06 ± 4.50), *Bacillus subtilis* (19.26 ± 5.84), *Candida* sp (21.17 ± 1.25), *Escherichia coli* (22.67 ± 4.52) amongst others showed considerable antimicrobial properties. *Salmonella spp* was observed to be the most resistant test organism used. The activities demonstrated by these extracts as presented in Table 6 showed varying minimum inhibitory concentration (MIC) against the tested organisms.The MIC recorded were (50 – 100) for *S. aureus, P. aeruginosa*, and *E. coli*. 25 – 100 and 12.5 – 100 were observed as the MICs for *Salmonella* spp and *C. albicans* respectively. In this work, we observed that the antimicrobial activities of the crude and fractions against the different test organisms were more on the Gram-negative test bacteria. This may be attributed to the difference in the permeability of the bacteria cell wall constituents between the Gram positives (thick cell wall) and Gram-negatives (thin cell wall). Moreover, the observed activity (broadspectrum) may be attributed to the bioactive phytoconstituents present in the extracts as detected by the phytochemical analysis. In this work, the observed inhibition of microbial growth of the test organism used was better than the controls used.

The results of the antioxidant activities demonstrated by the *Sphenostylis sternocarpa* crude extract and fraction in Table 7 can be said to be moderate, as only the ethyl acetate fraction was observed to demonstrate radical scavenging activities by both methods adopted. Moreover, the DPPH assay method revealed good antioxidant potentials demonstrated by the crude, and fractions of n-hexane, ethyl acetate, and butanol. This is in line with the result of Isaac-Bamgboye et al., [24], who observed an increase in the capacity of *S. sternocarpa* extract to scavenge DDPH. However, the capacity to scavenge free radicals by the most active fraction of ethyl acetate is not comparable with the activity by the control having an IC_{50} of 87.6 and 48 µg/mL as revealed by FRAP and DPPH assay methods respectively.

Preliminary detection of the phytoactive constituents revealed the presence of alkaloids, tannins, flavonoids, steroids, terpenoids, and saponins present in the crude and the fractions thus suggesting chemodiversity of plant secondary metabolites (Table 8). Similarly, Soetan et al., [13] detected the presence of alkaloids, flavonoids, and saponins in aqueous and acetone extracts of *Sphenostylis sternocarpa*. These phytoconstituents have also been detected and validated to be active and to have promising antimicrobial and antioxidant activities. Flavonoids are one of the most abundant phytoconstituents in plant and their action as antioxidants has been confirmed by several reports Kar, [25]; Ghasemzadeh and Ghasemzadeh, [26]. The capacity to inhibit the activities of the enzymes that generate free radicals, and function as a metal chelator is the basic antioxidant mechanism used by flavonoids, Soetan et al., [13]. In this work, the presence of saponins was detected in the ethyl acetate and aqueous fractions, which is an indication of the good antioxidant activities demonstrated by these two functions in comparison with other fractions and the crude. Similarly, reports by Chan et al., [27]; Chen et al., [28]; Soetan et al., [13] attributing the observed antioxidant activities of plant extracts to the presence of saponins corroborate our findings. The DPPH assay result indicates the potential of the active fractions to generate Diphenylpicrylhydrazine (reduced) through the conversion of DPPH accompanied by its decolorization.

4. Conclusions

The results obtained in the study further validate AYB as a potent underexplored medicinal plant with good antimicrobial and antioxidant potential. The detection of antimicrobial and antioxidant-related phytochemicals: alkaloids, terpene, saponins, and flavonoids are believed to be responsible for the observed activities hence AYB extract may be used in the preparation of preservatives. The huge phytodiversity within the *AYB* species reveals a source of the repertoire of phytoactive compounds and opens up a new dimension towards the discovery of new leads for the development of medicines for the treatment of infections and free radical-related disease. Secondary metabolites within

Compliance with ethical standards

Acknowledgments

The authors are sincerely grateful to Onwughalu Izuchukwu Camilus for defraying the publication fee

Disclosure of conflict of interest

Authors declare no competing financial interest.

References

[1] Okaiyeto K, Oguntibeju OO. African Herbal Medicines: Adverse Effects and Cytotoxic Potentials with Different Therapeutic Applications. Int J Environ Res Public Health. 2021;18(11):5988.

[2] Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014; 10; 4:177.

[3] Mukherjee P. W. Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals. New Delhi, India: Business Horizons Publishers. 2002

[4] Bodeker C., Bodeker G., Ong C. K., Grundy C. K., Burford G., Shein K. WHO Global Atlas of Traditional, Complementary and Alternative Medicine. Geneva, Switzerland: World Health Organization. 2005

[5] Bandaranayake W. M. "Quality control, screening, toxicity, and regulation of herbal drugs,'' in Modern Phytomedicine. Turning Medicinal Plants into Drugs eds. 2006

[6] Vitale M, Giosuè A, Sieri S, Krogh V, Massimino E, Rivellese AA, Riccardi G, Vaccaro O, Masulli M. Legume Consumption and Blood Pressure Control in Individuals with Type 2 Diabetes and Hypertension: Cross-Sectional Findings from the TOSCA.IT Study. Nutrients. 2023;6;15(13):2895.

[7] Sun XM, Ye HQ, Liu JB, Wu L, Lin DB, Yu YL, Gao F. Assessment of anti-diabetic activity of peanut shell polyphenol extracts. J Zhejiang Univ Sci B. 2018;19(10):764-775.

[8] George TT, Obilana AO, Oyeyinka SA. The prospects of African yam bean: past and future importance. Heliyon. 2020;11;6(11):e05458.

[9] Calapai G. European legislation on herbal medicines: a look into the future. Drug Saf. 2008;31 428–431

[10] Braun L. A., Tiralongo E., Wilkinson J. M., Spitzer O., Bailey M., Poole S. Perceptions, use and attitudes of pharmacy customers on complementary medicines and pharmacy practice. BMC Complement. Altern. Med. 2010.

[11] Anquez-Traxler, C. The Legal and Regulatory Framework of Herbal Medicinal Products in the European Union: A Focus on the Traditional Herbal Medicines Category. TherInnovRegul Sci. 2011:45, 15–23

[12] Olaiya CO, Idowu, PA, Karigidi KO. Antioxidative and antimicrobial activities of corn steep liquor anti-diabetic herb extracts. Annals Food Science and Technology. 2016; 17(2): 272-279

[13] Soetan, K.O, Olaiya, C. O, Karigidi, K.O. Comparative in vitro antioxidant activities of six accessions of African yam beans (Sphenostylisstenocarpa l.) Annals. Food Science and Technology. 2018;19: 3

[14] Xu BJ, Chang SKC. A Comparative Study on Phenolic Profiles and Antioxidant Activities of Legumes as Affected by Extraction Solvents. Journal of Food Science. 2007; 72(2).

[15] Adebajo J. Aladesanmi, E. O. Iwalewa, A. C. Adebajo, E. O. Akinkunmi, B. J.Taiwo, F. O. Olorunmolaand A. Lamikanra. Antimicrobial and Antioxidant activities of some Nigerian Medicinal plants. Afr. J. Trad. CAM .2007;4 (2): 173 – 184

[16] [16]. Ugochukwu M. Okezie, Peter M. Eze, Festus B.C. Okoye, Charles O. Esimone. (2022). Orthosporin, a major component of the fermentation product of Lasiodiplodiatheobromae- an endophytic fungus of Musa paradisiaca as a potential antimicrobial agent. NotulaeScientiaBiologicae. 2022; 14:2.

[17] European Committee for Antimicrobial Susuceptibility Testing (EUCAST). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution. the European Society of Clinical Microbiology and Infectious Diseases (ESCMID).2003

[18] Erhirhie, E.O., Ilodigwe, E.E. Ajaghaku, D.L..Umeokoli, B.O., Eze, P.M., Okoye, F.B.C. Antioxidant Activities of the Leaf Extract and Fractions of Dryopterisfilix-mas (L.) Schott could be attributed to the abundance of Polyphenol Compounds. Biology Medicine and Natural Product Chemistry.2020;9(1): 1 -6.DOI: 10.14421/biomedich.2020.91 .1 -6.

[19] Okezie, U.M., Okoli, O.A., Ajaegbu, E.E., Okoye, F.B.C., Esimone, C.O. Antioxidant Activities of Extracts Of Endophytic Fungi Isolated From Healthy Leaves OfCaricaPapaya". IJRBS. 2021;8(8), 48-56.

[20] Harborne JB. Textbook of phytochemical methods, 1st Edn, Champraan and Hall Ltd. London. Pp. 110-113. 1973

[21] Trease GE, Evans WC. Pharmacognosy. 2nd Ed., BaillierTindel and Macmillan publishers. 1989.

[22] Sofowora A. Phytochemical screening of medicinal plants and traditional medicine in Africa. 2nd Edition Spectrum Books Limited, Nigeria;. Drug Inf. J. 1993;150-156.

[23] Eruemulor, D. I. rbcl genetic relatedness and antimicrobial properties of african yam bean (sphenostylisstenocarpa) seeds. Covenant University, theses (2021)

[24] Bamgboye F.J.I, Victor N.E, Matthew O.O. In-vitro Antioxidant Capacity, Phytochemical Characterisation, Toxic and Functional Properties of African Yam Bean (Sphenostylisstenocarpa) SeedEnriched Cassava (Manihot esculenta) Product (Pupuru). European Journal of Nutrition & Food Safety. 2020;12(3): 84-98.

[25] Kar A (2007). Pharmacognosy and pharmacobiotechnology. New Age International, New Delhi. 2007; 332–600.

[26] Ghasemzadeh A, Ghasemzadeh N. Flavonoids and phenolic acids: Role and biochemical activity in plants and humans. Journal of Medicinal Plants Research. 2011; 5(31): 6697- 6703

[27] Chan KW, Iqbal S, Khong NMH, Ooi, D-J, Ismail M. Antioxidant activity of phenolics–saponins rich fraction prepared from defatted kenaf seed meal. LWT-Food Science and Technology. 2014;56(1): 181-186.

[28] Chen Y, Miao Y, Huang L, Li J, Sun H, Zhao Y, Yang J, Zhou W. Antioxidant activities of saponins extracted from Radix trichosanthis: an in vivo and in vitro evaluation. BMC Complementary and Alternative Medicine. 2014; 14:86-89.